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Gene gun-based nucleic acid immunization: elicitation of humoral and cytotoxic T lymphocyte responses following epidermal delivery of nanogram quantities of DNA

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Particle-mediated (gene gun) DNA transfer to the epidermis was evaluated for its ability to elicit humoral and cytotoxic T lymphocyte responses using decreasing quantities of plasmid DNA-based antigen expression vectors. Using plasmids encoding human growth hormone, human alpha-1-antitrypsin, and influenza virus nucleoprotein, strong immune responses were observed in mice following immunization with as little as 16 ng of DNA using an electric discharge gene delivery system. Significant antibody titers were observed against these antigens following a primary immunization, with responses rising dramatically following a boost. Increasing the DNA dose above 16 ng per immunization had little beneficial effect. In contrast to particle-mediated DNA delivery, intramuscular or intradermal inoculation required greater than 5000-fold more DNA to achieve comparable results. Data are also presented demonstrating that a simple, hand-held version of the Accell™ DNA delivery system, employing compressed helium as the particle motive force, achieves immune responses comparable to the traditional electric discharge device.

Keywords: Gene gun; DNA immunization; influenza; skin

Nucleic acid immunization involves the direct *in vivo* administration of antigen-encoding expression vectors for the purpose of eliciting antigen production and resultant specific immune responses[1–8]. This technology mimics live attenuated vaccines in that antigens are produced in their native conformation and are presented in the context of MHC class I and class II molecules to elicit cytotoxic cellular and humoral immune responses, respectively. This report is an extension of the study of Fynan *et al.*[4], in which it was demonstrated that particle-mediated (gene gun) delivery of an influenza virus hemagglutinin expression vector to the epidermis was superior to intramuscular inoculation for the elicitation of protective immunity in mice. Using three new antigen expression vectors, we demonstrate that the *Accell*® particle-mediated gene delivery system[1,2] elicits primary IgG responses following a single immunization with as little as 16 ng of plasmid DNA and that the respective titers can be boosted by 5- to 10-fold following a second immunization. The induction of similar responses via intramuscular inoculation required >5000-fold more DNA. We show that this difference in efficacy is likely due to the method rather than the site of delivery since intradermal inoculations also required >5000-fold more DNA to approach the titers obtained following particle-based DNA immunization of the skin. Finally, we demonstrate that a simple, hand-held gene delivery instrument that uses compressed helium as the particle motive force can achieve immunological results similar to those obtained using the more traditional and complex electric discharge device.

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MATERIALS AND METHODS

Expression vectors

pCMV-hGH contains the human cytomegalovirus (hCMV) immediate early promoter and encodes human growth hormone[1]. pCMV-hAAT encodes human alpha-1-antitrypsin (hAAT) and was constructed by inserting the 1.4 kb Not I fragment derived from pKPI-hAAT (Dr Kathy Ponder, Washington University, St. Louis, MO) into the pCMVβ vector (Clonetech, Palo Alto, CA)

following digestion with Not I to remove the beta-galactosidase gene. pCMV-NP was a gift of Dr Kari Irvine, National Cancer Institute, and contains the complete nucleoprotein (NP) coding sequence from influenza virus A/PR/8/34.

Particle-mediated DNA immunizations

Plasmid DNAs were accelerated into the abdominal epidermis of 6–8-week-old female BALB/c mice using the electronic *Accell*® gene delivery system (Agracetus Inc., Middleton, WI) as previously described[1,2,4], except that the skin was not pretreated in any way except for the removal of fur in the local area using clippers. All immunizations utilized a delivery energy of 15 kV. Epidermal immunizations employing a hand-held, helium-powered *Accell*® instrument contained 0.5 μg of DNA and 0.5 mg of 0.95 micron gold powder using a helium pressure setting of 400 p.s.i. The instrument is described in a recent PCT patent application[9].

Intramuscular and intradermal DNA inoculations

All intramuscular DNA immunizations involved injection of the quadriceps with 0.05 ml of 0.9% saline containing from 1 to 100 μg of plasmid DNA. All immunizations were administered invasively to anesthetized mice to guarantee proper placement of the inoculum. After anaesthetizing 6–8-week-old female BALB/c mice[1,2], a 1.5 cm incision was made through the skin along the inner thigh to expose the leg muscle groups. This was followed by injection of the DNA solution into the quadriceps and subsequent closure of the incision with surgical staples. Intradermal DNA immunizations were as described[8].

Antibody titer determination

Collection of blood samples and antibody titer determinations were as described previously[1,2]. For titration of influenza nucleoprotein IgG samples, 96-well plates were coated with detergent-disrupted influenza virus (50 μl per well of virus strain A/PR/8/34). In this case, sufficient virus was disrupted in lysis buffer (0.5 M Tris–HCl (pH 7.8), 0.6 M KCl, 0.5% Triton X-100) for 5 min at room temperature and then diluted with PBS to a final concentration of 4000 HA units per ml.

Cytotoxic T lymphocyte assays

Cytotoxic T lymphocyte responses to influenza nucleoprotein were measured as previously described[2] except that the synthetic nucleoprotein peptide (TYQRTRALV)[10] was substituted for the HIV-1 gp120 peptide.

RESULTS AND DISCUSSION

Figure 1 shows the results of two immunization trials comparing the endpoint IgG titers elicited to human growth hormone (hGH) and human α-1-antitrypsin (hAAT), respectively, following intramuscular (injection) or epidermal (gene gun) DNA immunizations using three different doses of DNA. In all cases, geometric mean titers following a single immunization were highest in the gene gun-immunized animals and the titers obtained were independent of the amount of DNA employed (1.6–0.016 μg DNA per immunization). The efficacy of the gene gun immunizations was further enhanced following a booster immunization in which the titers of all groups increased by 5- to 10-fold. In contrast, geometric mean titers in the intramuscular groups were considerably lower except for the group that received the 100 μg hAAT DNA immunizations. In the latter case, the responses were similar to those observed in the gene gun groups that received as little as 16 ng of DNA per immunization.

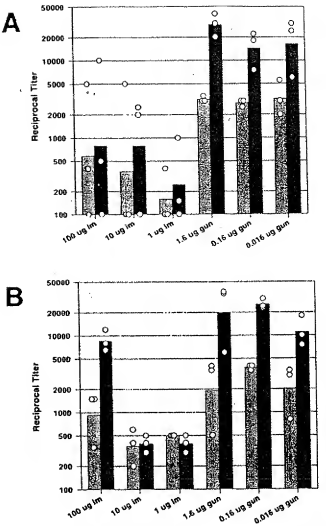

Figure 1 Six groups of three female BALB/c mice were each immunized on days 0 and 28 with the indicated amount of either pCMV-hGH or pCMV-hAAT DNA, either by intramuscular inoculation or via particle-mediated DNA delivery (gene gun) to the abdominal epidermis. Serum samples were collected on days 28 and 42 to measure primary and booster responses, respectively. Panel A, pCMV-hGH immunizations; Panel B, pCMV-hAAT immunizations. Gray bars, geometric mean titers following the primary immunization; solid bars, geometric mean titers following the booster immunization. Open circles show titers of individual mice

To determine if the enhanced immune responses following particle-mediated, intracellular delivery were due to the method rather than the site of delivery, a similar dose titration study was performed using an influenza nucleoprotein (NP) vector and substituting intradermal inoculation[8] for the more traditional intramuscular injection approach. IgG and cytotoxic T lymphocyte

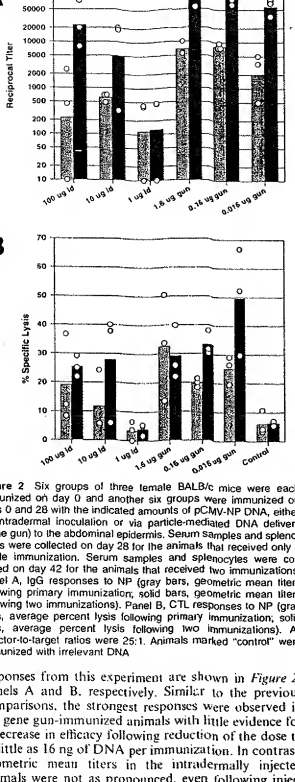

Figure 2 Six groups of three female BALB/c mice were each immunized on day 0 and another six groups were immunized on days 0 and 28 with the indicated amounts of pCMV-NP DNA, either by intradermal inoculation or via particle-mediated DNA delivery (gene gun) to the abdominal epidermis. Serum samples and splenocytes were collected on day 28 for the animals that received only a single immunization. Serum samples and splenocytes were collected on day 42 for the animals that received two immunizations. Panel A, IgG responses to NP (gray bars, geometric mean titers following primary immunization; solid bars, geometric mean titers following two immunizations). Panel B, CTL responses to NP (gray bars, average percent lysis following primary immunization; solid bars, average percent lysis following two immunizations). All effector-to-target ratios were 25:1. Animals marked "control" were immunized with irrelevant DNA

responses from this experiment are shown in *Figure 2*, Panels A and B, respectively. Similar to the previous comparisons, the strongest responses were observed in the gene gun-immunized animals with little evidence for a decrease in efficacy following reduction of the dose to as little as 16 ng of DNA per immunization. In contrast, geometric mean titers in the intradermally injected animals were not as pronounced, even following injection of as much as 100 μg of DNA. The cytotoxic T lymphocyte responses in these same animals were consistent with the IgG results in that dosage effects were not observed in the gene gun-immunized groups, but were seen following intradermal inoculation (*Figure 2b*). Control animals indicated in *Figure 2b* were immunized with irrelevant DNA. Additional controls in the CTL assay included coating target cells with irrelevant peptide in which background lysis values of 10% or less were

Table 1 NP-specific IgG titers following particle-mediated immunization with pCMV-NP DNA using the electric discharge and helium pulse instruments. Non-immunized control animals exhibited IgG titers of less than 1:10 (not shown)

Instrument	4 weeks post-prime	6 weeks post-boost
Helium pulse	4400	50 000
	5300	38 000
	3300	44 000
Electric discharge	4300	27 000
	3600	27 000
	4500	40 000

observed (not shown). NP-specific lytic activity was also shown to be associated with the $CD8^+$ T cell fraction following fractionation of lymphocyte subsets (not shown).

While this report and others have indicated that particle-mediated DNA immunization is an effective means of eliciting humoral and cytotoxic T lymphocyte responses in animal models, it has traditionally required an electronic instrument that is not practical for widespread clinical use. However, recent advances in gene gun technology have resulted in the development of a simple, inexpensive, hand-held *Accell*™ DNA delivery device that is better suited for particle-mediated gene delivery in clinical settings[9]. The effectiveness of this instrument for administration of nucleic acid vaccines is shown in *Table 1* in which the immune responses to NP were compared in animals immunized with 0.5 μg of the NP vector using either the electric discharge or the helium pulse devices. Essentially identical responses were obtained in both groups of animals, demonstrating a potential clinical role for particle-based DNA delivery technology in the area of vaccination and immunomodulation.

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REFERENCES

1 Eisenbraun, M.D., Fuller, D.H. and Haynes, J.R. Examination of parameters affecting the elicitation of humoral immune responses by particle bombardment-mediated genetic immunization. *DNA Cell Biol.* 1993, **12**, 791–797
2 Fuller, D.H. and Haynes, J.R. A qualitative progression in HIV-1 gp120-specific cytotoxic cellular and humoral immune responses in mice receiving a DNA-based gp120 vaccine. *AIDS Res. Human Retrovir.* 1994, **10**, 1433–1441
3 Tang, D.C., Devit, M.J. and Johnston, S.A. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992, **356**, 152–154
4 Fynan, E.F., Webster, R.G., Fuller, D.H., Haynes, J.R., Santoro, J.C. and Robinson, H.L. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proc. Natl Acad. Sci. USA* 1993, **90**, 11 478–11 482
5 Ulmer, J.B., Donnelly, S.E., Parker, S.E. *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 1993, **259**, 1745–1749
6 Wang, B., Ugen, K.E., Srikantan, V. *et al.* Gene inoculation generates immune responses against human immunodeficiency virus type 1. *Proc. Natl Acad. Sci. USA* 1993, **90**, 4156–4160

7 Davis, H.L., Michel, M.-L. and Whalen, R.G. DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody. *Hum. Molec. Genet.* 1993, **2**, 1847–1851
8 Raz, E., Carson, D.A., Parker, S.E. *et al.* Intradermal gene immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses. *Proc. Natl Acad. Sci. USA* 1994, **91**, 9519–9523
9 McCabe, D. PCT patent application. Publication date by 24 July 1995
10 Rotzschke, O., Falk, K., Deres, K. *et al.* Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature* 1990, **348**, 252–254